Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Extraction of phenolic compounds from dry and fermented orange pomace using supercritical CO_2 and cosolvents

Faber Ariel Espinosa-Pardo[a], *Vânia Mayumi Nakajima*[b], *Gabriela Alves Macedo*[b], *Juliana Alves Macedo*[b], *Julian Martínez*[a,*]

[a] College of Food Engineering, Food Engineering Department, University of Campinas, SP, Brazil
[b] College of Food Engineering, Food and Nutrition Department, University of Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 2 June 2016
Received in revised form 4 October 2016
Accepted 7 October 2016
Available online 17 October 2016

Keywords:
Supercritical fluid extraction
Orange
Phenolic compounds
Solid-state fermentation
Tannase
Antioxidant capacity

ABSTRACT

This work investigated the supercritical fluid extraction (SFE) of phenolic compounds from the pomace generated in the industrial processing of orange (*Citrus sinensis*) juice in Brazil. The effect of the biotransformation of the pomace, performed through solid-state fermentation using *Paecilomyces variotii*, in the phenolic content and antioxidant activity of the extracts was also evaluated. SFE was carried out at pressures of 15, 25 and 35 MPa and temperatures of 40, 50 and 60 °C, using pure ethanol and ethanol:water (9:1 v/v) as cosolvents. The extracts were evaluated in terms of global extraction yield, total phenolic content (TPC), phenolic profile by HPLC, and antioxidant activity through DPPH and ORAC assays. Additionally, a low pressure extraction was performed (Soxhlet) for comparison to SFE, using ethanol as solvent. The results of SFE showed that high pressures improved the global extraction yield, which ranged from 2.01 to 2.62%, and TPC (18–21.8 mg GAE/g dry extract). Nevertheless, the increase of pressure decreased the antioxidant activity of the extracts. The use of ethanol 90% as cosolvent enhanced the extraction of antioxidant compounds. The biotransformation process improved the TPC and provided extracts with higher antioxidant activities by both DPPH and ORAC assays.

1. Introduction

Several works indicate that the consumption of fruits has beneficial effects in the prevention of cardiovascular, circulatory and neurological diseases due to their anti-inflammatory, anti-allergic, antithrombotic and antimicrobial activities (Espinosa-Pardo et al., 2014; Lagha-Benamrouchea and Madani, 2013; Balasundram et al., 2006). The biomolecules responsible for the chemoprotective effects are found not only in the fruit pulp, but also in the leaves, peels and seeds. *Citrus* fruits have gained interest due to their high content of these biocompounds, especially flavonoids. Flavonoids belong to the group of phenolics, among which flavanones are the most abundant group in *Citrus* fruits (Madeira et al., 2014; Ferreira et al., 2013; Barros et al., 2012). Naringin and hesperidin are the major flavanones found in tissues and peels of *Citrus* fruits, exhibiting many health benefits due to their anti-inflammatory and anticarcinogenic effects (Madeira et al., 2014; Ferreira et al., 2013; Meiyanto et al., 2012; Nazari et al., 2011; Park et al., 2008).

Brazil is currently the world's largest producer of oranges, above countries such as United States, China and Mexico, representing about 30% of the world's production of fresh fruit. In 2012 the production was around 18 million tons, and about 80% of this production was destined to process concentrated juice (FAO, 2012; Investe São Paulo, 2013). As a result of this industrialization, large amounts of residues composed by peels, seeds and membranes are discarded, which represent almost half the weight of the fresh fruit (Mamma and Christakopoulos, 2014; Khan et al., 2010; Benelli et al., 2010). These by-products can be used to obtain pectin and feed for livestock. However, residues of *citrus* are a

* *Corresponding author.*
E-mail addresses: julian@unicamp.br, jmartinez94@gmail.com (J. Martínez).
http://dx.doi.org/10.1016/j.fbp.2016.10.002

potential source of phenolic compounds (M'hiri et al., 2015), specifically glycosylated flavonoids with biological activity.

The antioxidant compounds obtained from the orange pomace and their biological activities have already been extensively studied (Lagha-Benamrouchea and Madani, 2013; Barros et al., 2012; Chen et al., 2012; Khan et al., 2010; Li et al., 2006a,b) using conventional and/or low pressure extraction methods. Nevertheless, there are more efficient and environmentally friendly extraction processes, especially when dealing with the recovery of natural compounds that exhibit antioxidant properties. Supercritical fluid extraction (SFE) takes advantage of the properties of fluids over their critical points to selectively extract soluble components from different biological matrices. In the supercritical state, solvents have properties of both liquids and gases, e.g., low viscosity and high diffusivity and density, which are convenient in extractive processes (Rosa et al., 2008; Brunner, 1994). Supercritical carbon dioxide is recognized as an adequate solvent to extract bioactive compounds because it is non-explosive, nontoxic, readily available, selective and can be easily separated from the final extract, preserving its biological properties (Cavalcanti et al., 2016; Garcia-Mendoza et al., 2015; Espinosa-Pardo et al., 2014; Macías-Sánchez et al., 2007; Cavero et al., 2006).

Phenolic compounds may be the compounds that most contribute to the antioxidant potentials of foods (Balasundram et al., 2006; Parr and Bolwell, 2000), and their antioxidant activity depends on the number and position of the hydroxyl groups related to the carboxyl functional group (Cipriano, 2011; Balasundram et al., 2006; Robards et al., 1999). The capacity of phenolic acids to scavenge free radicals increases with their hydroxylation degree. Different flavonoid profiles can be obtained from solid matrices through solid-state fermentation process using fungi, yeast or bacteria able to hydrolyze ester and depside bonds of phenolic compounds through the tannin acyl hydrolase (tannase) production (Bhoite and Murthy, 2015; Ferreira et al., 2013; Madeira et al., 2013; Chamorro et al., 2012).

In this context, the present work aimed to investigate the SFE of phenolic compounds from orange (*Citrus sinensis*) pomace that results from the industrial processing in the production of juice, in order to obtain extracts with high antioxidant activity. The influence of temperature, pressure and cosolvent in SFE was evaluated. Conventional Soxhlet extraction was carried out for comparison with SFE, and the effect of the solid-state fermentation of the pomace, using *Paecilomyces variotii*, in the modification of the phenolic profile was investigated.

2. Material and methods

2.1. Raw material and sample preparation

Orange pomace composed by peel (flavedo and albedo), pulp (juice sac residue), rag (membranes and cores) and seeds was supplied by the company CPKelco, Limeira, SP, southeastern Brazil. The material was provided as dry residue and ground in a 600 W domestic blender (Philips, R12008/81, SP, Brazil) in order to reduce the particle size and increase the contact surface with the extraction solvent. Next, the material (named in this work as "dry orange pomace") was kept in absence of light at −18 °C in a domestic refrigerator, until the extractions were performed.

2.2. Fermented orange pomace

In order to produce the biotransformed orange pomace the methodology described by Madeira et al. (2012) was followed, with some modifications. The *P. variotii* strain was isolated (Laboratory of Food Biochemistry, UNICAMP) and selected to produce tannase and modify the orange phenolic profile. The fungus strain was deposited at the Brazilian Collection of Environmental and Industrial Micro-organisms (CBMAI, number 1157).

The fungal inoculum was obtained by the sporulation of the fungal strain on PDA (Potato dextrose Agar) medium and incubated at 30 °C for 72 h. Then, the spores were suspended in distilled water at a minimum concentration of 9×10^6 spores/mL.

For the fermentation, 10 g of orange pomace was added to 10 mL of distilled water in 250 mL Erlenmeyer flasks. After sterilization in autoclave, the flasks were inoculated with 1 mL of the previous inoculum suspension and incubated at 30 °C and 90% relative humidity (Climate Chamber 420 CLD, Nova Etica, SP, Brazil) for 72 h. Then, the fermented orange pomace was homogenized, dried for 4 h at 60 ± 2 °C in an air circulation oven (model 320 SE, Fanem, SP, Brazil) and stored at −18 °C in a domestic refrigerator until its use.

2.3. Material composition and particle characterization

The dry and fermented orange pomaces were characterized to determine their proximate compositions. Analyses of moisture and ash were performed according to AOAC methods (AOAC 934.06 and 942.05, respectively). The total lipid content was determined by the AOAC method 963.15 using petroleum ether as solvent, and crude protein was determined by the semi-micro-Kjeldahl procedure (AOAC 970.22).

The ground material was characterized by size classification in a vertical vibratory sieve shaker (Bertel Metallurgic Ind., Ltd., SP, Brazil) and the geometric mean particle diameter (d_{mg}) was calculated according to ASAE standards (1998), using 50 g of sample and standard sieves of Tyler series (WS Tyler No. 9; 10; 14 20; 28; 35; 48; 65; 80 and 100, Mentor, USA).

The real density (ρ_r) of the particles was determined by helium gas pycnometry, and bulk density (ρ_a) was obtained as the ratio between the mass used to form the particle bed inside the extraction column and the volume of the extraction bed. The porosity (ε) of the extraction bed was calculated using Eq. (1).

$$\varepsilon = 1 - \left(\frac{\rho_a}{\rho_r}\right) \quad (1)$$

2.4. Supercritical fluid extraction (SFE) with cosolvent

SFE from dry orange pomace was performed in a dynamic extraction unit illustrated in Fig. 1, which was assembled in the Laboratory of High Pressure in Food Engineering—LAPEA/DEA/FEA-UNICAMP.

The SFE unit consists of a 50 mL jacketed extraction column (Autic, Campinas, Brazil); a cooling bath (Marconi, MA-184, Piracicaba, Brazil); a pneumatic pump (Maximator, M-111 CO_2, Nordhausen, Germany) to achieve the required pressure; a heating bath (Marconi, MA-126, Piracicaba, Brazil) to reach the process temperature; a micrometer valve (Autoclave Engineers, 10VRMM2812, Erie, USA) where CO_2 is depressurized; and a flow totalizer and manometers to monitoring the process. A cosolvent pump (PU-2080 plus, Jasco, Easton, USA) was connected to the extraction line at pre-established flow rate, to mix the cosolvent with CO_2 before entering the extraction column.

The SFE procedure consisted of initial static period of 20 min followed by a dynamic extraction time of 75 min. The mass of raw material used for the extractions was 10 g, and the mass ratio between solvent and orange pomace (S/F) was kept constant at 124 ± 2 kg solvent/kg pomace. The pressures and temperatures evaluated in SFE were 15, 25, 35 MPa and